Farm Exposure as a Differential Risk Factor in ANCA-Associated Vasculitis

P. Willeke[1]*, B. Schlüter[2], C. Sauerland[3], H. Becker[1], S. Reuter[1], A. Jacobi[4], H. Schotte[1]

1 Department of Medicine D, Section of Rheumatology and Clinical Immunology, University Hospital Münster, Münster, Germany, **2** Centre for Laboratory Medicine, University Hospital Münster, Münster, Germany, **3** Institute of Biostatistics and Clinical Research, University of Münster, Münster, Germany, **4** Division of Rheumatology and Clinical Immunology, Brandenburg Medical School, Neuruppin, Germany

* willeke.peter@uni-muenster.de

Abstract

Objective

To investigate the association of farm exposure and the development of ANCA-associated vasculitis (AAV).

Methods

One hundred eighty-nine well defined patients with AAV (n = 119 with granulomatosis with polyangiitis [GPA], n = 48 with microscopic polyangiitis [MPA], n = 22 patients with eosinophilic granulomatosis with polyangiitis [EGPA]) and 190 controls (n = 119 patients with rheumatoid arthritis, n = 71 with large vessel vasculitis) were interrogated using a structured questionnaire. Factors investigated were occupation, farm exposure, contact to different livestock, participation in harvesting, residence next to a farm, MRSA status, and contact to domestic pets at disease onset or ever before. The odds ratio (OR) and 95% confidence interval [95%CI] were calculated for each item.

Results

Univariate analysis revealed a strong association of AAV with regular farm exposure; OR 3.44 [95%CI 1.43–8.27]. AAV was also associated with regular contact to cattle 4.30 (1.43–8.27), pigs 2.75 (1.12–6.75) and MRSA carriage 3.38 (1.11–10.3). This association was stronger in the subgroup of GPA patients. OR in this group for farm exposure was 4.97; [2.02–12.2], for cattle 6.71 [95% CI 2.19–20.7], for pigs 4.34 [1.75–10.9], and MRSA carriage 5.06 [1.62–15.8]). There was no significant association of MPA or EGPA with these parameters.

Conclusion

A significant association between farm exposure or farm animal exposure and AAV especially in the subgroup of patients with GPA has been identified. This suggests that these entities are distinct and have different triggers for the immune process.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic necro-
tising vasculitis predominantly affecting small vessels. AAV comprises granulomatosis with
polyangiits (GPA, Wegener's), microscopic polyangiitis (MPA), and eosinophilic granuloma-
tosis with polyangiitis (EGPA) [1]. The annual incidence of AAV as a group is estimated at
about 10–20 patients per million [2]. The causes of AAV are poorly understood and their
pathophysiological mechanisms remain uncertain [3].

In a recent genome-wide association study of patients with AAV a genetic contribution to
the pathogenesis has been confirmed [4]. Beside the genetic susceptibility microbial pathogens
and environmental factors have been implicated in the pathogenesis of AAV [5].

Various potential environmental risk factors have been suggested [4, 6]. Particularly, occu-
pational exposure to different agents has been associated with the development of AAV [7, 8].
Several case-control studies found a positive association between crystalline silica exposure and
other inhaled agents with GPA and MPA [6]. Also differences in the geographic distribution
have been reported suggesting that environmental factors may play a role in the pathogenesis
of the disease [9]. In addition, an association with bacterial infection could be suggested in
AAV [10].

Farming has been reported as a risk factor for the development of several autoimmune dis-
eases [7, 11]. An association of farming and AAV has also been reported [12, 13]. However,
there are inconsistent data about farming as a risk factor for AAV. In a recent Swedish case-
control study no significantly association of farming or animal exposure with the development
of AAV or GPA respectively could be detected [14]. The information about the disease and the
occupation of patients in this study was obtained from registry data of inpatient care and may
thus be less precise than data directly acquired using questionnaires.

The aim of the present study was to investigate in a large hospital based case-control study
whether farming or farm exposure represent risk factors for the development of AAV in the
north western part of Germany.

Materials and Methods

AAV patients and controls

The study protocol was approved by the local independent ethics committee (Ethikkommis-
sion der Ärztekammer Westfalen-Lippe). All patients and controls provided written informed
consent before participating in the study. This study was conducted in compliance with Good
Clinical Practices and the Declaration of Helsinki and was approved by the local independent
ethics committee.

We included patients with different AAV subtypes. GPA was diagnosed according to the
American College of Rheumatology (ACR) criteria and the criteria adapted from the 2012
revised Chapel Hill Consensus Conference (CHCC) [1, 15]. MPA was diagnosed according to
the CHCC definition [1]. EGPA was diagnosed according to the ACR criteria and the CHCC
definition [1, 16]. In addition, the EMA algorithm for classification of AAV was used to further
correlate clinical and laboratory findings with the different AAV subgroups [17]. A biopsy was
performed if possible. In cases in which no biopsy was available the diagnosis was defined by
typical clinical manifestations and positive results for antibodies against proteinase-3 (PR3) or
myeloperoxidase (MPO). ANCA-negative patients were included if a histologic confirmation
of vasculitis was available.

As controls we included patients with rheumatoid arthritis (RA) and patients with large ves-
sel vasculitis (LVV, i.e. Takayasu arteritis and giant cell arteritis) or polymyalgia rheumatica

(PMR). LVV and PMR are closely related disorders [18]. Diagnosis was based on the American College of Rheumatology (ACR) 1987 criteria for RA and the criteria adapted from the 2012 revised CHCC [1, 19].

Patients and controls were selected from our university hospitals inpatient or outpatient department of Rheumatology.

In order to avoid significant differences in the geographic area of residence patients and controls were matched for the geographic catchment area at disease onset.

Data collection, laboratory analysis

Each chart was carefully reviewed for the accuracy of the diagnosis as well as for clinical and laboratory details which includes disease onset and ANCA findings.

Disease activity of AAV was assessed by the Birmingham Vasculitis Activity Score version 3 (BVAS v.3) [20]. A relapse was defined as an increase in the disease activity. Remission was defined as an absence of clinical disease activity as indicated by a BVAS v.3 score of 0.

Carriage of MRSA was analysed in AAV patients and controls by chart review or results from nasal or pharyngeal swab culture for MRSA, respectively. In all inpatients a standardized screening for MRSA was performed at admission to our hospital (i.e. swabs from nose, throat, axilla, and groin).

Antibodies to PR3 and MPO were tested with a sensitive capture fluorescent enzyme immunoassay (Phadia EliA) performed on an ImmunoCAP 250 analyzer (Thermo Fisher Scientific, Germany).

Questionnaire

Occupation and farm exposure was evaluated by a structured interviewer-administered questionnaire. All questionnaires were performed as direct interview either at presentation in our hospital or via telephone. The interview was performed by one and the same unblinded investigator (PW).

Patients were asked about their occupation at disease onset and during the working lifetime before. Further, a comprehensive history about farming and farming related parameters was requested. A translated version of the questionnaire can be found in the online attachment (S1 File). All questions were related either to the time of disease onset or to the time ever before (defined as > 1 year before disease onset).

The items analysed were occupation, farm exposure, participation in harvesting, exposure to cattle, pigs, horses or poultry. Our query further included current and past exposure to pets. This was performed separately for dogs, cats, and other pets. In addition subjects' history of residence was requested. Participants were asked whether they lived on or directly next to a farm at disease onset or ever before.

Also the frequency of exposure was asked (i.e. regular defined as several times per month; occasionally defined as several times per year or rarely defined as once per year or less). All questions about exposure to different animals were related only to direct contact to these animals.

Statistics

We compared the whole cohort of AAV patients with the control group. In addition, disease subtypes (i.e. GPA, MPA, and EGPA) were compared to the control group.

Data were analysed using SPSS20 and Medcalc 13.0 (Medcalc Software, Mariakerke, Belgium).

Univariate binary logistic regression for each exposure of interest was used to calculate odds ratios (OR), 95% confidence intervals (95%CI) and the p-values from the Wald-test.

Continuous variables were expressed as mean ± standard deviation (SD), categorical variables were summarized by absolute und relative frequencies. For categorical variables the comparisons were calculated by Fisher's exact test or Chi-square test and for continuous variables using the Mann–Whitney U-test. The result was considered significant in case of the two-sided $p < 0.05$.

Results

Patient characteristics

A total of 189 patients with AAV were enrolled. In 119 patients GPA was diagnosed, 48 patients had MPA, and 22 patients suffered from EGPA. The control group consisted of 190 individuals, including 119 patients with RA and 71 patients with LVV/PMR (Fig 1). Clinical characteristics of AAV patients and controls are given in Table 1.

There were no significant differences in age or gender between AAV patients or controls. Also we found no significant differences in the geographic area of residence between AAV patients and control groups or the distance to our hospital.

Questionnaire

A direct interview for the questionnaire was performed in 83% of AAV patients and 80% in the control group during presentation at our hospital. In the other cases the query was performed as telephone interview. Only 2 patients considered for the control group were not willing to participate in the study. If deceased (n = 3 in the AAV group) the interview was performed by proxy (spouse or direct descendant). All other AAV patients performed the questionnaire personally.
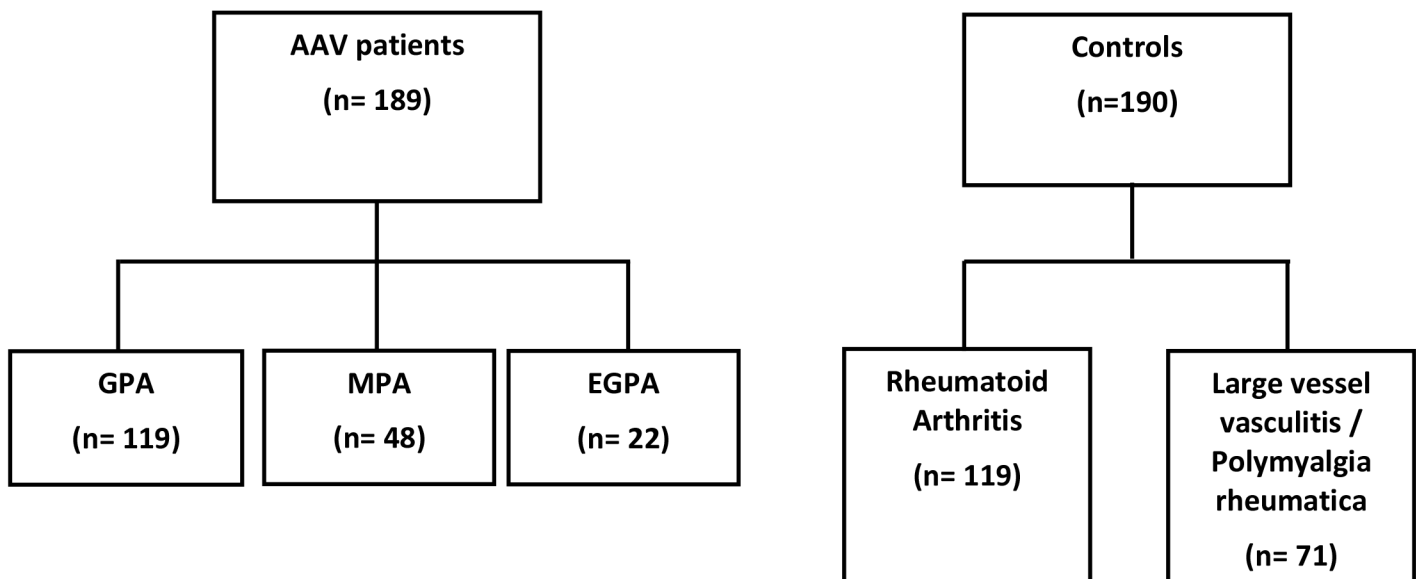

Fig 1. Schematic diagram showing the number of study participants in each group. AAV = ANCA associated vasculitis; GPA = granulomatosis with polyangiitis (Wegener's); MPA = microscopic polyangiitis; EGPA = eosinophilic granulomatosis with polyangiitis.

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Table 1. Demographic and clinical characteristics of patients with AAV:

	AAV	GPA	MPA	EGPA	Control
Total	189	119	48	22	190
Age at disease onset (years)	51.2 ± 15.8	50.8 ± 16.0	54.7 ± 13.1	44.4 ± 16.4	51.6 ± 16.6
Gender (male/ female)	100 / 89	61 / 58	30 / 18	9/ 13	89 / 101
Distance to hospital (km)	46.2 ± 26.1	48.7 ± 27.9	45.4 ± 25.2	35.2 ± 22.1	46.3 ± 39.0
Biopsy performed, n (%)	163 (86.2)	101 (85)	43 (87.5)	19 (86.4)	-
Relapse, n (%)	93 (49.7)	68 (57.1)	16 (33.3)	9 (45)	-
Sinusitis, n (%)	133 (70.4)	105 (88.2)	7 (14.6)	21 (95)	-
Pulmonary involvement, n (%)	116 (61.4)	72 (60.5)	22 (45.8)	22 (100)	-
Renal involvement, n (%)	118 (63.1)	72 (60.5)	44 (91.7)	2 (10)	-
Polyneuropathy, n (%)	47 (24.9)	27 (22.7)	6 (12.5)	14 (63.6)	-
CNS Involvement, n(%)	16 (8.4)	10 (8.4)	2 (4.1)	4 (22.7)	-
Skin involvement, n (%)	29 (15.3)	17 (14.3)	5 (10.4)	7 (31.8)	-
ANCA positive (%)	163 (87.7)	115 (95.0)	47 (97.9)	3 (15)	-
PR3-ANCA–positive, n (%)	117 (62.6)	112 (94.1)	4 (8.3)	1 (5)	-
MPO-ANCA positive, n (%)	46 (24.6)	1 (0.8)	43 (89.6)	2 (10)	-

GPA = granulomatosis with polyangiitis (Wegener's); MPA = microscopic polyangiitis; EGPA = eosinophilic granulomatosis with polyangiitis; MPO = myeloperoxidase; PR3 = proteinase 3; BVAS v.3 = Birmingham Vasculitis Activity Score Version 3.

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Occupation

Occupations of AAV patients and controls were grouped according to the following occupation-groups: students, housewives, pensioner, retailer, office workers, IT workers, industrial workers, civil servants, manual worker, farmer, miscellaneous, vehicle driver/ motor mechanic, health professional, electrician/ electrical engineer, metal worker, concrete worker, teacher, unemployed, or army/ fire department worker). We found no significant differences in the occupation groups between AAV patients and controls (S1 Fig). In particular, this held true also for main-occupation farmers.

We could not detect an increased number of occupation associated with silica exposure as suggested by Lane et al. (i.e., coal miner, coal delivery, sandblaster, mine worker, quarry worker, baker, dental worker, painting and decorating, or working in the construction industry) in the AAV compared to the control group [12]. Though, a detailed survey about qualitative and quantitative silica exposure during business or outside main occupation was not separately performed.

Farming and farm related parameters

The questionnaire revealed that farm exposure was by no means restricted to main-occupation farmers. Indeed, from the 22 AAV patients with regular farm exposure 12 had so outside the main occupation. These patients were regularly working on a farm as additional business.

The prevalence of the different parameters analysed in the questionnaire are given in Table 2.

The OR and the 95% CI are shown in Table 3. AAV as disease group was significantly associated with regular farm exposure (p = 0.005). An even higher odds ratio was found for GPA and regular farm exposure in the subgroup analysis (p< 0.001). In contrast, no significant association of MPA or EGPA with farming was found. In addition, we detected a significant association of AAV with regular contact to cattle or pigs. Again the association was restricted to GPA

Table 2. Results of the questionnaire with prevalence for different parameters requested in AAV patients and controls at disease onset:

	AAV (%)	GPA	MPA	EGPA	Control (%)	RA	LVV/PMR
Total	189	119	48	22	190	119	71
Farm exposure	22 (11.6)	19	3	0	7 (3.68)	7	0
Residence next to farm	14 (7.41)	8	5	1	7 (3.68)	5	2
Harvesting	14 (7.41)	11	3	0	6 (2.63)	6	0
Livestock animals	27 (14.3)	23	3	1	14 (7.37)	12	2
Cattle	16 (8.47)	15	1	0	4 (2.11)	4	0
Pig	18 (9.52)	17	1	0	7 (3.68)	6	1
Horse	7 (3.70)	4	3	0	6 (3.16)	5	1
Poultry	8 (4.23)	7	0	1	9 (4.74)	7	2
Pets:	54 (28.6)	34	15	5	49 (25.8)	35	14
Dog	39 (20.6)	27	10	2	37 (19.5)	27	10
Cat	15 (7.93)	8	4	3	11 (5.79)	7	4
Other	10 (5.29)	6	4	0	5 (2.63)	4	1
Ever farm exposure [1]	24 (12.7)	15	5	4	20 (10.5)	12	8

[1] = before disease onset.

GPA = granulomatosis with polyangiitis (Wegener's); MPA = microscopic polyangiitis; EGPA = eosinophilic granulomatosis with polyangiitis;

RA = rheumatoid arthritis; LVV = large vessel vasculitis; PMR = polymyalgia rheumatic.

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patients whereas no association was found for MPA or EGPA patients (Table 3). GPA was significantly associated with participation in harvesting.

Different OR and the 95% CI of GPA patients compared to non-GPA patients with AAV (i.e. MPA; EGPA) are shown in Fig 2. The forest plot indicates high OR in patients with GPA compared to non-GPA patients with AAV in several farming related parameters. No

Table 3. Odds ratio and 95% CI for different parameters of farming or animal exposure in AAV patients and controls:

	AAV vs. controls OR (95%CI); p	GPA vs. controls OR (95%CI); p	MPA vs. controls OR (95%CI); p	EGPA vs. controls OR (95%CI); p
Farm exposure	3.44 (1.43–8.27); p = 0.006 *	4.97 (2.02–12.2); p< 0.001*	1.74 (0.43–7.01); p = 0.43	0.54 (0.03–9.80); p = 0.73
Ever farm exposure	1.24 (0.66–2.32); p = 0.51	1.22 (0.60–2.50); p = 0.58	0.99 (0.35–2.78); p = 0.98	1.98 (0.58–6.14); p = 0.21
Harvesting	2.45 (0.92–6.52); p = 0.07	3.12 (1.12–8.69); p = 0.029 *	2.04 (0.49–8.49); p = 0.32	0.69 (0.04–12.7); p = 0.80
Livestock animals:	2.01 (1.01–3.97); p = 0.046 *	3.01 (1.48–6.12); p = 0.002*	0.84 (0.23–3.04); p = 0.79	0.29 (0.02–5.10); p = 0.40
Cattle	4.30 (1.41–13.1); p = 0.010 *	6.71 (2.19–20.7); p = 0.001*	0.98 (0.11–9.06); p = 0.99	1.01 (0.05–19.5); p = 0.99
Pig	2.75 (1.12–6.75); p = 0.025 *	4.34 (1.75–10.9); p = 0.002 *	0.06 (0.07–4.63); p = 0.59	0.60 (0.03–10.8); p = 0.73
Horse	1.17 (0.39–3.56); p = 0.78	1.06 (0.29–3.84); p = 0.92	2.04 (0.49–8.49); p = 0.33	0.69 (0.04–12.7); p = 0.80
Poultry	0.89 (0.34–2.35); p = 0.81	1.26 (0.45–3.47); p = 0.66	0.19 (0.01–3.44); p = 0.26	0.46 (0.03–8.30); p = 0.60
Pets:	1.15 (0.73–1.81); p = 0.54	1.15 (0.69–1.92); p = 0.59	1.31 (0.65–2.61); p = 0.45	0.84 (0.29–2.41); p = 0.76
Dog	1.07 (0.65–1.78); p = 0.78	1.21 (0.69–2.12); p = 0.49	1.09 (0.50–2.38); p = 0.83	0.46 (0.10–2.07); p = 0.31
Cat	1.40 (0.63–3.14); p = 0.41	1.17 (0.46–3.01); p = 0.75	1.48 (0.45–4.86); p = 0.52	2.87 (0.73–11.3); p = 0.13
Other	2.07 (0.69–6.17); p = 0.19	1.96 (0.59–6.59); p = 0.28	3.36 (0.87–13.0); p = 0.08	0.82 (0.04–15.4); p = 0.89
Residence next to farm	2.09 (0.82–5.30) p = 0.12	1.88 (0.66–5.34); p = 0.23	3.03 (0.92–10.0); p = 0.07	1.37 (0.16–11.8); p = 0.77

GPA = granulomatosis with polyangiitis; MPA = microscopic polyangiitis; EGPA = eosinophilic granulomatosis with polyangiitis; OR = odds ratio 95%

CI = 95% confidence interval

* = statistically significant.
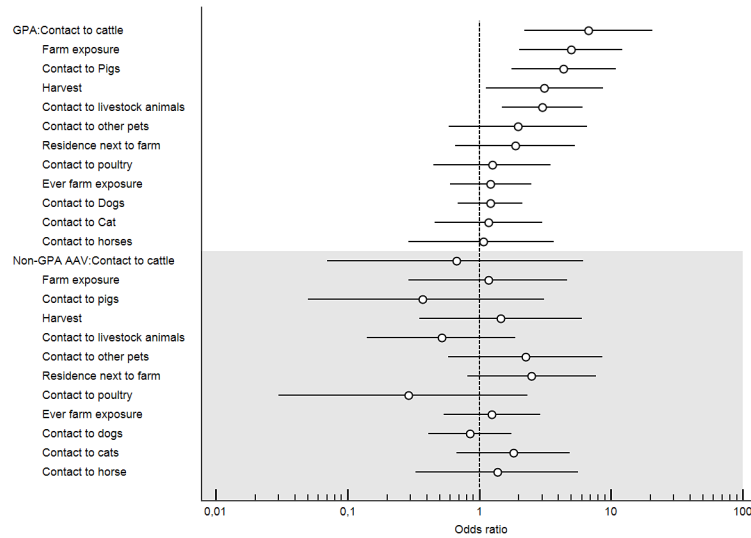
doi:10.1371/journal.pone.0137196.t003

Fig 2. Forest Plot. The bars represent the odds ratio (circle) and 95% confidence interval for the association of GPA with different parameters (upper part) and for non-GPA patients with ANCA-associated vasculitis (lower grey part). AAV = ANCA-associated vasculitis; GPA = granulomatosis with polyangiitis (Wegener's).

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significant associations of non-GPA patients and the items analysed in our questionnaire could be detected.

We have also evaluated the results of the questionnaire by separating patients into PR3-or MPO-ANCA positive and ANCA negative patients. The prevalence in the three patients-groups for the different items analysed were given in S1 Table. However, this analysis did not reveal an increase in the prevalence.

We could not detect significant associations of domestic pets with AAV or the subgroups.

We found no association of AAV with exposure to the different parameters ever before disease onset (). Also there was no significant difference in patients with occasional or rare frequency of farm exposure between the two groups or between the subgroups and controls (S2 Table).

Patients with farm exposure were mainly exposed to more than one type of livestock animal or other farm associated parameters analysed (i.e. participation in harvesting).

MRSA status

MRSA status was not available in all patients (Table 4). Carriage of MRSA was tested in 164 patients with AAV (86.8%) and 121 patients in the control group (63.7%). We found an association of AAV with MRSA carriage (OR 3.38 [95%CI: 1.11–10.31]; p = 0.033). An even higher odds ratio was found in patients with GPA (OR 5.06; [95%CI: 1.62–15.78]; p = 0.005). Six out of fifteen positively tested patients had worked as farmer and had regular contact to cattle or pig (40%).

We found no association of MPA or EGPA with MRSA carriage compared to control. In addition, we found no significant difference in clinical manifestations between GPA patients carrying MRSA compared to GPA patients without MRSA carriage. Also no difference in the BVAS at disease onset was found (16.1 ± 7.9 vs. 17.5 ± 6.6; p = 0.27). In addition, no difference in the relapse rate was found between these groups (58.8% vs. 46.0%; p = 0.44) during the follow up period of 2.7 ± 1.5 years.

Table 4. Prevalence of methicillin resistant staphylococcus aureus in patients with ANCA associated vasculitis (AAV) and controls as well as odds ratio and 95% CI for MRSA in AAV patients and controls:

	AAV	GPA	MPA	EGPA	Controls	RA	LVV
Total	189	119	48	22	190	119	71
testing performed (%) [1]	164 (86.8)	101 (84.9)	44 (91.7)	19 (86.3)	121 (63.7)	73 (61.3)	47 (66.2)
MRSA (%) [2]	17 (10.4)	15 (14.9)	1 (2.3)	1 (5.0)	4 (3.33)	3 (4.11)	1(2.13)
	AAV vs. controls	GPA vs. controls		MPA vs. controls		EGPA vs. Controls	
OR (95%CI)	3.38 (1.11–10.33)	5.06 (1.62–15.78)		0.67 (0.07–6.20)		2.21 (0.24–20.74)	
p	p = 0.032	p = 0.005		p = 0.72		p = 0.48	

[1] = number and percentage of total in each disease group.

[2] = number and percentage of MRSA positive patients of tested patients.

GPA = granulomatosis with polyangiitis; MPA = microscopic polyangiitis; EGPA = eosinophilic granulomatosis with polyangiitis; RA = rheumatoid arthritis; LVV = large vessel vasculitis.

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Subgroup analysis

We found no associations with farming and a specific clinical manifestation (i.e. presence or absence of renal involvement or respiratory involvement, local manifestations) or the predominance of specific disease stages for GPA (i.e. local, early systemic, generalised or severe). One GPA patient with farm exposure was negative for PR3- and MPO-ANCAbut demonstrated a typical histology. All other GPA patients with farm exposure were positive for PR3 antibodies. AAV patients with farm exposure did not exhibit a higher BVAS at disease onset (17.4 ± 6.2 vs. 17.2 ± 6.7, p = 0.70). Also these patients did not show a significantly higher rate of relapses (63.6% vs. 49.7%, p = 0.32).

Discussion

The pathogenesis of AAV has not yet been fully elucidated. Recent genome wide genetic association studies revealed a strong genetic link to the development of AAV [4, 5]. Our result suggests that beside the genetic contribution environmental factors may play an important role for the pathogenesis of AAV.

Since GPA, MPA, or EGPA may have different triggering factors the different entities were compared individually to the control group. Our data clearly demonstrate that the association of farming and farming related items is restricted to the GPA entity of AAV and does not involve MPA or EGPA.

The rural region surrounding our centre is characterised by a high density of livestock farming [21]. However, the control group was from the same geographic area of residence and had a significantly lower percentage of farm exposed patients. An increase of farm exposure in GPA patients due to different catchment areas could be excluded.

A possible relationship between farming and the development of AAV has been described previously [12–14]. Lane et al. detected a stronger association of farming at the time of first clinical manifestation with MPA compared to GPA. On the other hand farming during the working lifetime was significantly associated with GPA but not with MPA. However, they included only 47 patients with GPA, 12 patients with MPA, and 16 patients with EGPA. In accordance with our results they also found no association of farming with EGPA [12].

In the study of Knight et al. 2288 cases with GPA and a 10 fold larger control group was included. They found no significant association of farm-working with GPA in this large case-control study. However, the data about occupation was obtained from the Swedish Register of

inpatient care and not obtained by a survey or direct interview. Occupational exposure can be different between people listed for the same occupation. Also exposure to farms or farm animals outside the occupation was not considered. In addition, the diagnosis of GPA was based on the national hospital registry referring to the discharging doctor's medical diagnosis. This data can be incorrect to a considerable extent [14].

Also a questionnaire may not cover all aspects of farm exposure or different items associated with farming but it is much more efficient in identifying patients with farm exposure or contact to livestock animals than registry data. Several patients in our study had regular contact to farms or livestock animals during their additional business although their main occupation was not farmer or farm worker.

Lee at al. reported different clinical manifestations in GPA patients with or without high farming activity [13]. They found no association with farming in patients with localised GPA compared to patients with high farming activity who exhibited pulmonary and renal involvement in their small cohort of eight patients. We have not detected an association with different clinical manifestations in GPA patients with or without farm exposure. We could further not detect an association of farming with disease severity or the incidence of a relapse.

In accordance with the findings of Lane et al. we observed that exposure to livestock animals was stronger compared to other farm associated items (i.e. participation in harvesting) [12]. However, since most individuals were exposed to more than one item it is not possible to clearly differentiate between specific farming exposures. The strongest association of GPA was found with contact to cattle and pigs. Interestingly, we found no association of GPA with contact to horses or poultry. It is not possible to further explain this specific finding based on our data. Several factors in the care of cows or pigs may be responsible for this observation. This includes animal feeds or the use of antibiotics.

The prevalence of MRSA in GPA patients was very high. The MRSA prevalence rate (including colonisations) in Germany varies approximately from 1 to 3% [22].

We found a strong association of GPA with MRSA colonisation compared to controls. Nasal colonisation with staphylococcus aureus (staph. aureus) has been implicated in GPA pathogenesis and activity [23]. Especially the persistent colonisation of the nasal passage with these bacteria may play a pivotal role in the pathogenesis [24]. Persistence of staph. aureus colonisation is more likely in methicillin-resistant strains of this organism. It has been shown that MRSA positive farmers are more likely to be persistently colonized than transiently contaminated [25].

Staph. aureus has been described as a strong inducer of neutrophil extracellular trap (NET) formation [26]. NETs are extracellular lattices of chromatin, histones, and granule enzymes extruded by neutrophils [27]. Increased NET formation in AAV has been associated with the development of ANCA, since NETs increase the antigen exposure of PR3 or MPO to the immune system [28]. Especially chronic presence of NETs due to MRSA may facilitate stimulation of the production of ANCA [29, 30].

However, we cannot definitely substantiate whether this coincidence of MRSA carriage with GPA represents a causal relationship or not [31]. Nasal colonisation or contamination with livestock associated MRSA can be found in up to 86% of all farmers in Germany [32]. Thus, our finding of an increased MRSA colonisation can also be an epiphenomenon associated with farming.

Beside staph. aureus several other causative organisms have been associated with ANCA formation and GPA [33, 34]. Among these many bacterial pathogens are particularly common in livestock animals (i.e. campylobacter or helicobacter) [35, 36]. Thus, other livestock associated pathogens may be responsible for the association we found.

Also other factors associated with farming or the care of farm animals may play a role. An association of pesticide exposure with the development of rheumatoid arthritis or other auto-immune diseases has also been described [11]. Also an association of contact to pesticide with AAV has been reported [37]. However, this parameter was not analysed in our study. We detected an association of GPA with participation in harvesting. Perhaps crop exposure may represent a risk factor for GPA.

We found no association of silica exposure with the development of AAV. Occupational exposure to silica was not increased in AAV patients or the subgroups compared to control. An increased exposure to silica has been assumed in farmers participating in manual harvesting of potatoes, tobacco or peanuts but not in other crops [38]. Although an exposure to silica in the form of agricultural dust could not be excluded none of our patients was involved in harvesting potatoes, tobacco or peanuts. Our patients participated in grain harvesting only.

Although we could not isolate a specific trigger associated with farming we have identified farming and additional parameters associated with farming as a risk factor for AAV as a disease group, specifically in the sub group of patients with GPA. Additional structured investigations are needed to further substantiate farming as a risk factor for the development of AAV or GPA, respectively, and to identify specific determinants responsible for the pathogenesis. The results represented here may provide a concept for further analysis regarding the association of farming with the development of GPA.

Supporting Information

S1 Fig. Number of occupations at disease onset in patients with ANCA-associated vasculitis and controls. There was no significant difference in the occupation groups between ANCA-associated vasculitis patients and controls.
(TIF)

S1 File. Translated version of the questionnaire.
(PDF)

S1 Table. Results of the questionnaire separately for patients with PR3-ANCA or MPO-ANCA and ANCA negative patients.
(DOCX)

S2 Table. Results of the questionnaire.
(XLSX)

Author Contributions

Conceived and designed the experiments: PW HS. Performed the experiments: PW. Analyzed the data: PW CS BS SR AJ. Wrote the paper: PW BS CS HB SR AJ HS.

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